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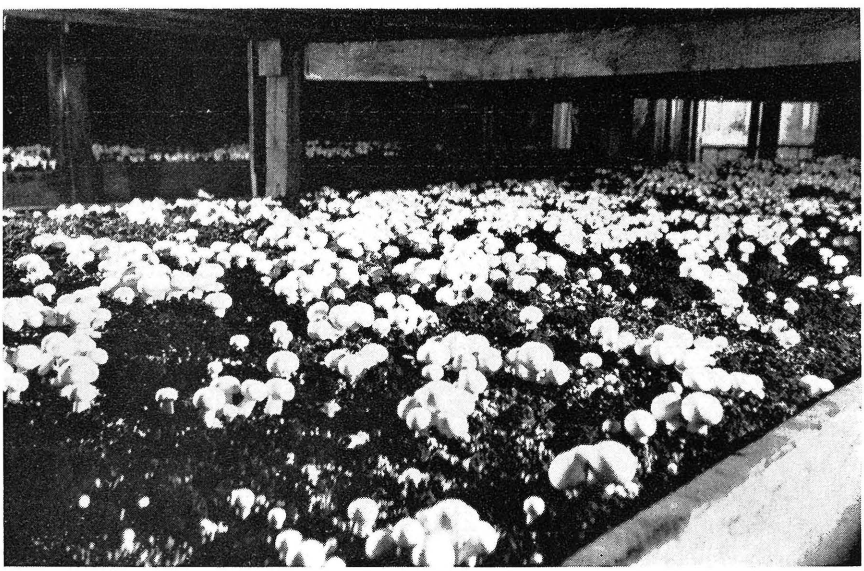

OHIO AGRICULTURAL EXPERIMENT STATION

Wooster, Ohio

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ON THE COVER

Fig. 1.—Mushroom bed in a commercial house near the peak of production. Note mushrooms in all stages of maturity.

EFFECT OF SUPPLEMENTARY B-COMPLEX VITAMINS ON YIELDS AND VITAMIN CONTENT OF MUSHROOMS

IRA C. HOFFMAN and CHARLES H. HUNT[1]

It has been the experience of commercial producers of the cultivated mushroom, *Agaricus campestris,* that after picking heavily for a few weeks, the yield begins to drop off rapidly. The heavy picking period varies from 8 to 10 weeks depending upon the conditions within the beds. Considerable study has been made to find out why, but so far the exact cause or causes have not been fully determined.

Composted horse manure has been found by experience to be the best natural material with which to fill the beds, and investigators have been trying for years to find some way to lengthen the heavy picking period, thereby, increasing the yield of mushrooms. Various materials, both organic and inorganic, have been added to the manure as supplements. At times the yields have shown some increases and at other times with similar treatments they have not. This suggested that some factor might cause the drop in yield other than the usual substances found in chemical analysis, because at the end of the crop there are still sufficient nutrients left to indicate that the mushrooms harvested had not depleted the original supply very much.

It is generally known that horse manure contains considerable amounts of the vitamin B-complex, but it was not known whether or not the mushroom fungus can synthesize all or part of the various components of the B-complex. The objects of this study are first, to determine whether or not additions of the various components of the B-complex would stimulate greater production of mushrooms, second, whether or not a deficiency of any one, or all, of the various components could account for the rapid reduction in yields after the first few weeks of picking, and third, whether or not additions of any one, or all of the various components of the B-complex would increase the amount of these vitamins within the mushrooms following their application to beds while in

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production. In addition to the pure chemical components of the B-complex, several organic supplements which contain large quantities of the B-complex will be tried.

In preliminary tests (4), (5), (6), thiamine, vitamin B_1 was used. The vitamin was dissolved in water and sprinkled evenly over duplicate portions of a bed in a commercial mushroom house. Records showed that the average yields of the treated areas were about 15 percent greater than those of similar areas treated with an amount of water equivalent to that used in the solution (3).

REVIEW OF LITERATURE

Mushrooms have been used as food since ancient times and have been relished by peoples around the world. Native varieties differ widely from place to place, but in recent times the common cultivated species, *Agaricus campestris* has been introduced throughout Europe and America. Mushrooms use large quantities of lignin and water insoluble protein fractions (15) from the compost in their growth. It is also thought that they utilize hemicellulose, but probably not cellulose. Waksman (16) analyzed samples of fresh and spent mushroom compost and found that there was still a "fair" amount of these materials present after a normal cropping period. Rettew, Gahm and Divine (10) and Waksman (16) found that the ratio of hemicellulose: to cellulose: to lignin: to water insoluble protein in spent mushroom compost was similar or even slightly higher than in recently composted manure before the crop was grown.

Investigators concentrated for a time on studying the effects on mushroom production of adding to the compost different amounts of the major and minor elements regarded as essential to produce normal growth in green plants. Edwards (2), Lambert and Ayers (7), Pizer (8), Sinden (11), Stoller (12) and Treschow (14) studied different major and minor elements and found the results variable and insignificant. Lambert and Ayers (7) and Treschow (14) used water insoluble protein as a compost supplement. The results with these were also variable and not conclusive. Watts (17) reported results of analyses of spent mushroom manures and fresh horse manures. He stated, "All in all, the spent mushroom manures are somewhat richer in nitrogen and potash, and much richer in phosphoric acid than an equal weight of fresh horse manure." The mineral portions are concentrated by a loss in organic matter. It would seem, then the sudden dropping off in yield after a few weeks of heavy picking must be due to something other than a large loss in organic matter or a deficiency of mineral nutrients, since considerable amounts remain in the spent compost after picking has been completed.

Anderson and Fellers (1) report mushroom protein as "partially incomplete", or similar in nature to gliadin of wheat and hordein of barley. Mushrooms were also found to be an excellent plant source of nicotinic acid and riboflavin, a good source of pantothenic acid, and a fair source of vitamins, B_1 C and K.

Knight and Frazier (6) state "It generally is assumed that corn steep liquor enhances penicillin production because of a specific organic constituent, but during a study of the physiology of *Penicillin chrysogenum* it was found that the inorganic constituents of corn steep liquor played an important role." The solids were dried and ashed at 1400° F. for 4 to 5 hours and different increments were mixed with the synthetic media on which the penicillin was grown. The amount of penicillin increased as the amount of ash increased in the medium and with the time of incubation. So in this case increases in yield of penicillin at least were attributed to inorganic materials, but from organic sources.

EXPERIMENTAL MATERIALS

Mushroom Houses: The experiments were conducted in two commercial mushroom raising establishments[2] at North Olmsted, Ohio and in an especially equipped room at the Ohio Agricultural Experiment Station. The commercial mushroom houses were relatively new and quite modern in design. The beds were about 6 ft. wide, 6 in. deep, and 35 ft. long. The beds were arranged in tiers with one bed above another leaving enough space between to permit filling the beds, watering and picking the mushrooms. The houses were equipped with steam heating, watering and gravity ventilating systems so the conditions for best mushroom production could be controlled. (See Fig. 1).

The mushroom house at the Experiment Station was converted from an underground vegetable storage room. (See Fig. 2). It was fitted with beds in two tiers similar to those in the Dean and Paddock houses, except that the beds were about 4 ft. 4 in. wide, 6 in. deep, and 48 ft. long. The house was also equipped with a thermostatically controlled hot water heating system and with watering and forced-air ventilating systems.

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[2]Samuel Dean and Sons, and Roger N. Paddock, Paddock Fishley Greenhouse Co. to whom the authors wish to extend their personal thanks for excellent help and cooperation.

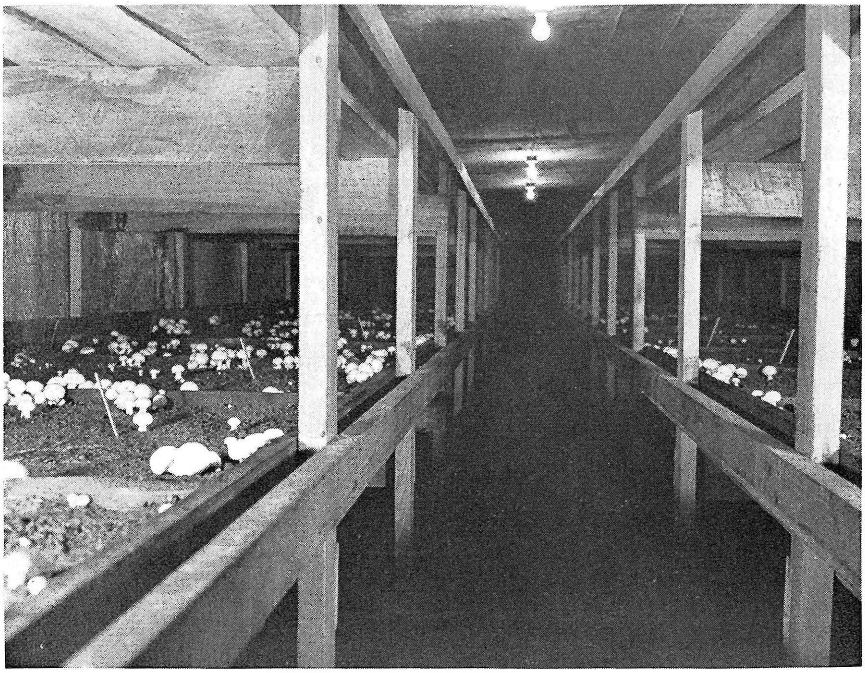

Fig. 2.—Interior of experimental mushroom house. Note different yield responses in the plots due to different vitamin treatments.

Growth Media: Horse manure for these experiments was gathered from riding stables in the metropolitan area of Cleveland and contained approximately equal portions of wheat straw and animal manure. The manure for the Experiment Station tests was secured from one of the cooperating commercial growers and was composted in the same ricks with the manure for that grower's houses. In this way it received proper heating and mixing. In making the ricks, the manure was placed in piles 6 ft. to 8 ft. wide, 6 ft. high and long enough to contain all the manure needed to fill all the beds in the cooperators' houses. As the manure was piled all lumps were shaken apart and the straw and animal manure evenly distributed throughout the rick. Enough water was added to dampen the rick thoroughly while it was being piled. Then the ricks were allowed to stand for the first heating period. The heat was generated by the natural fermentation of the manure and the temperature rose to 130° – 140° F. in the center of the pile in 8 to 10 days. The ricks were then repiled twice more at intervals of 8 to 10 days each in such a way that the outside of the piles was thrown to the inside of the new ricks. This was done so that during the second and third heating periods all portions of the manure would receive similar heat treatments. During the heating periods the manure lost its fecal odors and the straw became dark chocolate brown in color. The straw also became pliable enough to break off easily when twisted lightly with the hands. When the manure reached this condition it was deemed ready for the beds. As soon as the cooperator had filled his beds, enough of the composted manure was brought to the station house to fill the beds. It was put in immediately and packed lightly to exclude large air spaces and to prevent drying out. As soon as the beds in the commercial houses and the Experiment Station house were filled the houses were closed and the temperature was allowed to rise, since fermentation was still going on actively in the composting manure.

The temperature in the beds rose to 130° to 140° F. in about 5 days and it was maintained for 2 days more. The purpose of this heating was (1) to bring the compost to approximately uniform moisture content; (2) to kill harmful bacteria and fungi that may have still remained in the manure; (3) to complete the changes in the compost so that the mushroom spawn could obtain its food more readily; and (4) to drive insects and mites to the surface of the compost where they could be killed most easily. When this heating had reached its peak, the Experiment Station house was opened long enough to apply Cyanogas fumigant to kill all insects and mites. The gas was confined for a day after which the house was opened, ventilated, and the beds were allowed to begin to cool. The cooperating commercial houses were put through the final heating period and fumigated in a similar manner.

In about 4 days when the compost had cooled to 80° F. in all three houses the beds were spawned similarly with spawn secured from the same mushroom supply house. The temperatures of the houses were reduced gradually to 70° F. and the spawn left to grow through the compost. The beds were watered as needed to keep the compost in the proper moisture condition. The temperature of the compost was kept at 70° F. for a week to induce the spawn to grow rapidly through the compost. When examination of the beds showed that the mycelium of the mushrooms had penetrated all parts of the compost the temperature was gradually reduced to 60° F. and the beds were ready for casing.

Casing the beds was simply spreading soil uniformly about 1 inch thick over the surface of the beds. The soil was similar in all 3 houses and consisted of neutral loam brought in from fields. The soils were run through soil shredders, screened to remove trash, and then steam sterilized to kill weed seeds, insects, and all bacteria and fungi that might attack the mushrooms. The casing soils were prepared several weeks in advance and then stored in dry protected places until needed.

VITAMIN SUPPLEMENTS

The vitamins were supplied in water soluble and insoluble forms. The soluble ones were in the pure crystaline form used in controlled biological research. The insoluble ones were organic forms prepared for agricultural use.

Soluble Vitamins: Thiamine was tried first and when it was found to increase the yields of mushrooms, riboflavin, nicotinic acid, pantothenic acid, pyridoxine and biotin were added to the study.

These vitamins were secured from biochemical supply companies from ingredients especially selected for nutritional investigations. These vitamins belong to the B-complex and were water soluble. They were furnished as thiamine hydrochloride, riboflavin, nicotinic acid, calcium pantothenate, pyridoxine hydrochloride and biotin.

Organic Supplements: The organic supplements were BY-100, BY-500, milorganite, cyclone products and Kapost, all of which contained large amounts of the B vitamins. BY-100 and BY-500 were dried fermentation solubles from butyl acetonic fermentation of corn. Their riboflavin contents had been adjusted to uniform values of 100 and 500 micrograms per gram of dry material, respectively. These products were normally used as supplements within animal feeds.

Milorganite and cyclone products, commonly used as fertilizers, were prepared commercially from sewage by the Milwaukee, Wisconsin disposal plant.

In addition, milorganite contained twenty minerals, nitrogen and considerable amounts of finely divided organic matter. Cyclone products were the most finely divided fraction of milorganite. They were separated from the coarser particles of milorganite when the milorganite was passed through the cyclones while being prepared for use as fertilizer. Cyclone products were similar in composition to milorganite. They contained a small amount of chaff, which came from the grain used in the malt industry. The chaff was added to the cyclone products to keep the cyclone products in good physical condition for drilling as a fertilizer.

TABLE 1.—B-Complex values of dried butyl acetonic fermentation solubles of corn*

B Complex Vitamins	Micrograms per Gram, Dry Weight	
	BY-100	BY-500
Riboflavin	100.00	500.00
Pantothenic Acid	148.00	153.00
Nicotinic Acid	78.00	81.30
Folic Acid	6.50	6.65
Para Amino benzoic Acid	17.40	17.70
Biotin	0.33	0.33
Pyridoxin	5.00	5.18
Thiamine	0.50	.56
Inositol	3682.00	3620.00
Choline	2041.00	2052.00
B_{12}	0.21	0.21

*These analyses were supplied by the manufacturer—Commercial Solvents Corporation, Terre Haute, Indiana.

Kapost was the commercial name for a product made from composted cotton seed hulls. The compost was dried and ground. No vitamin analysis was available, but it was reported by the manufacturer to contain considerable amounts of the B-complex vitamins.

Since these materials contained appreciable amounts of the B-complex vitamins, it was deemed worth trying them as supplements to mushroom composts to increase yields.

TABLE 2.—Analysis of milorganite*

B-Complex Components	Micrograms per Gram, Based on Dry Weight
Riboflavin	16.5
Nicotinic acid	126.0
Pantothenic acid	118.0
Pyridoxine	6.0
Biotin	4.0

*Analysis by the manufacturer—Milwaukee Sewerage Commission, Milwaukee, Wisconsin.

EXPERIMENTAL METHODS

Plot Arrangement: In the cooperators' mushroom houses, the beds were about 6 feet wide, 6 inches deep, and nearly 55 feet long. They were arranged in tiers with one bed above another leaving enough space between to permit filling the beds, water and picking the mushrooms. The beds chosen for the treatments were in the interior of the houses and of sufficient height (4½′) from the ground to be surrounded by uniform air temperature. They were also of convenient height for easy application of the vitamins and picking the mushrooms. The plots for treatment were 25 square feet, 6 feet by 4.2 feet in area and arranged at random in duplicate.

In the station house the beds were constructed in two tiers with two beds each, because the house was narrow and low. The tiers were so arranged that the tiers stood close to the walls with a 2-foot walk along the middle of the house. The beds were 4 feet 4 inches wide, 48 feet long and 6 inches deep. The plots were 3 feet wide and reached across the bed. They were separated by a strip of asphalt roofing 4 feet 4 inches long and 6 inches wide which stood on edge between them and reached completely across the bed from front to rear. (See Fig. 2). The area of each plot was 13 square feet, and the treatments were arranged at random in duplicate in the station house.

Soluble Vitamins: Preliminary tests were made with different amounts of thiamine and increased yields of mushrooms were secured. Then it was decided to study the effects of such B-complex components as riboflavin, nicotinic acid, pantothenic acid, pyridoxine and biotin in addition to thiamine. Dr. Roland M. Bethke and Dr. Charles H. Hunt of the Animal Science Department, O.A.E.S. had much experience with these vitamins in feeding animals and in conference with the senior author were able to suggest the respective amounts of the vitamins probably needed to produce characteristic effects peculiar to each one. Doctor Hunt analyzed samples of mushrooms from each treatment at regular intervals during the crop to find if these vitamins increased the amounts of the vitamins within the mushrooms, when supplied to mushroom beds while in production.

The amounts of the vitamin materials to be applied per application per square foot of plot area in these experiments are listed in Table 3.

TABLE 3.—Amounts of the B vitamins applied per square foot of bed area

Thiamine Hydrochloride	.50 mgm
Riboflavin	1.00 mgm
Nicotinic Acid	12.50 mgm
Calcium Pantothenate	3.75 mgm
Pyridoxine Hydrochloride	1.00 mgm
Biotin	.004 mgm

Each of the B-complex vitamins was weighed out in the appropriate amounts either singly or put in different combinations at the rates listed above to make up the desired treatment and dissolved in one quart of tap water. The solution was sprinkled evenly over the plots and followed immediately by a regular irrigation with tap water which was large enough presumably to percolate through the casing soil and carry most of the vitamins down into the composted manure where most of the mushroom mycelia were located. This procedure was followed in the two commercial houses and the Station house to duplicate plots whenever the chemical vitamins were applied.

ORGANIC SUPPLEMENTS

The organic supplements were weighed out and were applied uniformly over the proper plots at the time the beds were filled with the freshly composted manure. They were carefully mixed with the manure at that time so they would go through the final heating period, and be sterilized with the manure. The organic materials were applied at the following rates:

TABLE 4.—Rates of applying the organic supplements

Materials	Pounds per Square Foot	Pounds per Plot, 25 Square Foot
BY-500*	.15	3.75
BY-100	.50	12.50
Milorganite	.50	12.50
Cyclone products	.50	12.50
Kapost	.50	12.50

*BY-500 was so much richer in riboflavin than the others it was applied at approximately one-third the rate.

After the final heating and the beds had cooled sufficiently, the plots were spawned and later cased in a manner similar to the rest of the beds in the commercial and Station houses.

Sampling: After the plots came into production, samples of mushrooms were taken from all treatments, weighed, dried, ground and stored in air-tight containers until analyzed. Samples were collected from the commercial and station plots at two-week intervals, and just before the next application of the vitamin solutions was made so that none of the chemicals would be adhering to the outside of the mushrooms and introduce errors in the analyses. In all cases the mushrooms were selected carefully so that they were similar in size, age (before the veils opened) and condition to have them as uniform as possible.

Chemical Analyses: The dried mushrooms were analyzed for the various vitamins by Dr. Charles H. Hunt, using the methods (18) approved and adopted by food nutrition chemists. All results were expressed on the dry weight basis in micrograms per gram of product.

Results: Preliminary experiments were made to determine the amount of thiamine to use per square foot of bed area, whether more than one treatment should be made during the crop, and when the treatments should start.

The first experiment was made to determine whether different amounts of the vitamin would affect the yield of mushrooms. Plots were laid out in duplicate and applications of thiamine were made once to each plot at the rates indicated in Table 5 after the bed had been cased. The total yields are given in Table 5. It was found in this experiment that the yield of mushrooms increased as the concentration of thiamine was increased, up to 0.5 milligram per square foot of bed area.

TABLE 5.—The effect of the amount of thiamine on the yield of mushrooms, one applications

Treatment in mgm per Square Foot	Yield per Square Foot	Difference Due to Treatments	Percentage Gain Due to Treatment
	lb.	lb.	%
1. Check—water	1.20	--	----
2. 0.001	1.40	.20	16.6
3. 0.01	1.50	.30	25.0
4. 0.1	1.60	.40	33.3
5. 0.5	1.79	.59	49.1

The next experiment had to do with finding the time when the application should be started for highest yields. Plots were arranged in duplicate and thiamine at the rate of .5 milligram per square foot was supplied in solution at each application. In treatment No. 2, Table 6, the thiamine solution was applied to the compost just before the bed was cased. In treatment No. 3 the solution was applied to the plots just after the bed was cased. Treatment No. 4 was varied to include applications of the vitamin at the same rate to the plots both before and after casing the bed to see if two such treatments would produce larger increases in yield. The yield data are in Table 6. Increases in yield in Treatments 2 and 3 were large enough to show that applications of thiamine were practical this early in the mushroom picking season. When applications at the same rate were made both before and after the plots were cased, the yields increased to 32 percent over the check plot. However, twice the amount of thiamine had been used. This suggested that additional applications could be made during the crop and perhaps expect further increases in yields. Subsequent tests showed increased yields up to 6 applications per crop. The applications were also made at two-week intervals and at the rate of 0.5 milligrams per square foot of bed area each time. At this point the yield was more than 49 percent greater than the untreated checks. The experiment was stopped after 13 weeks of picking by the end of the crop.

TABLE 6.—Effect of time of applying thiamine on mushroom yields

Treatment	Average yield per square foot	Difference due to treatment	Percentage gain
	lb.	lb.	
1. Check—water	2.5		
2. Thiamine on compost before casing only	2.7	+.2	8.0
3. Thiamine after casing only	2 8	+.3	12.0
4. Thiamine on compost and on casing soil	3.3	+.8	32.0

Since increases in yield of such magnitudes with thiamine were reached, the experiments were broadened to study the effects of certain other members of the vitamin B-complex in addition to thiamine on yield and quality of the mushrooms. Nicotinic acid, riboflavin, pyridoxine, pantothenic acid and biotin were tried separately along with thiamine and the results are presented in Table 7.

TABLE 7.—The effect of certain B-vitamins on the yield of commercial mushrooms. One application in each case

Treatment	Average yield per square foot	Difference due to treatment	Percentage increase
	lb.	lb.	
Check	1.63		
Thiamine	1.85	.22	13.5
Nicotinic acid	2.11	.48	29.4
Riboflavin	1.79	.16	9.8
Pyridoxine	1.71	.08	4.9
Pantothenic acid	1.90	.27	16.6
Biotin	1.86	.23	13.8

Fig. 3.—The larger yield of mushrooms in the plot on the left was due to applications of thiamine and nicotinic acid combined. The plot on the right was an untreated check.

This experiment was run in one of the cooperating commercial mushroom houses and the picking season was somewhat shorter than usual. This accounts for the smaller yield per square foot and also for the lower percentage increases due to the treatments than were obtained in a later experiment in the Station house (see Table 8). It does show, however, that each of these vitamins when applied alone can increase mushroom yields appreciably.

Another experiment was set up at the Station experimental house to compare the effects on yields of certain combinations of the vitamins with the vitamins applied singly and with untreated checks. The vitamins were weighed out in increments as stated previously whether they were applied singly or mixed as combinations and applied in duplicate plots. (Figs. 2, 3 and 4). The vitamins applied singly were thiamine, riboflavin, nicotinic acid and pantothenic acid. The combinations made of two vitamins mixed together were thiamine + riboflavin; thiamine + nicotinic acid (see Fig. 2); and riboflavin + nicotinic acid. The combinations with three vitamins were riboflavin + nicotinic acid + pantothenic acid; and thiamine + nicotinic acid + pantothenic acid. There was one treatment containing all four of these vitamins mixed together in the same amounts and applied in solution like the others. The results are presented in Table 8.

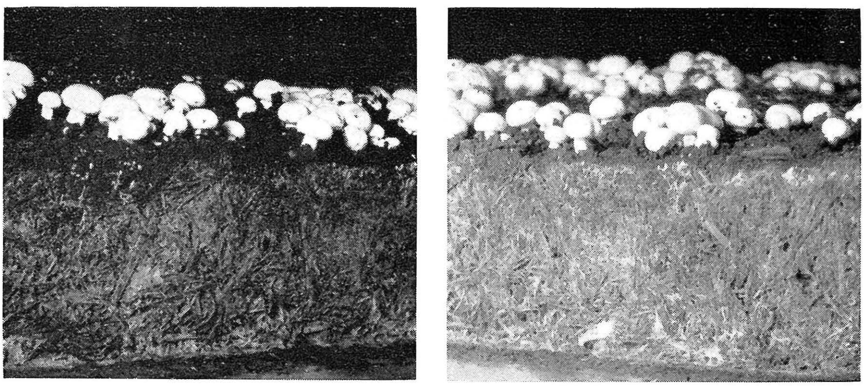

Fig. 4.—Portions of two mushroom beds prepared similarly, except the one on the right was treated once with the complete mixture of B-vitamins before casing while the one on the left was untreated. Note the greater run of spawn and more mushrooms in the treated bed six weeks after picking started.

TABLE 8.—Effect on yields of supplementing mushroom manure compost with B-complex vitamins, singly and in various combinations. Ohio Agricultural Experiment Station

Treatment	Average yield per plot*	Average yield per sq. ft.	Increase due to treatment	Percentage increase
	lb.	lb.	lb.	
Check	11.5	.88		
Thiamine	14.3	1.10	.22	25.0
Riboflavin	14.4	1.10	.22	25.0
Nicotinic Acid	13 3	1.02	.14	15.8
Pantothenic Acid	14.6	1.12	.24	27.2
Thiamine + Riboflavin	16.7	1.23	.35	39.7
Thiamine + Nicotinic Acid	19.7	1.51	.63	71.6
Riboflavin + Nicotinic Acid	16.8	1.29	.41	46.6
Riboflavin + Nicotinic Acid + Pantothenic Acid	20.5	1.57	.69	78.4
Thiamine + Nicotinic Acid + Pantothenic Acid	18.4	1.41	.53	60.2
Thiamine + Riboflavin + Nicotinic Acid + Pantothenic Acid	17.4	1.34	.46	52.3

*Area of each plot was 13 square feet.

The vitamins applied singly produced increases in yields about as in previous experiments. The yield produced by nicotinic acid was somewhat lower than the rest, but all produced considerable increases over the checks. The combinations consisting of two vitamins produced considerably more mushroom than the single vitamins and much more than the checks. The two combinations consisting of three vitamins mixed together produced still greater increases over all the treatments whether the vitamins were applied singly or in twos, except thiamine + nicotinic acid. The yield of this combination was in about the same order of magnitude as the yields of the combinations in threes. The treatment consisting of all four vitamins in combination produced a yield of 52.3 percent greater than the check plot, but not quite so great as for combinations of vitamins in threes. Whether this drop in yield was caused by a total vitamin content which was too concentrated was not determined. It was observed that the yields of the plots increased steadily as the number of vitamins increased until there were three vitamins in the mixture. The combination of four vitamins caused a slight drop in yield in this experiment.

The question was raised whether yields of mushrooms would be increased if vitamins were applied to beds that had been in produceion for several weeks. In order to answer that question the following experiment was set up.

DELAYED APPLICATIONS OF B-VITAMINS

In the mushroom house at the Station the crop was started in the usual way and left without treatments until 24 pickings (4 weeks) had been made. Then treatments of the chemical forms of the vitamins in solution were started and applied at ten-day intervals to duplicate plots for the next 24 pickings (4 weeks). The vitamins were applied singly, in certain combinations of twos and threes and all four combined. The results are given in Table 9.

TABLE 9.—Effect of delayed B-vitamin treatments on the late yields of mushrooms.* Ohio Agricultural Experiment Station

Treatments	Average yield of plots. 24 pickings preceding treatments	Average yield of plots. 24 pickings after treatments started	Difference due to treatments	Percentage increase
	lb.	lb.	lb.	
Check	4.3	2.8	—1.5	—53.8
Thiamine	4.2	7.0	2.8	40.0
Riboflavin	4.3	5.6	1.3	23.2
Nicotinic Acid	3.0	5.9	2.9	49.1
Pantothenic Acid	3.9	6.4	2.5	39.0
Thiamine + Riboflavin	4.0	7.8	3.8	48.7
Riboflavin + Nicotinic Acid	4.3	6.0	1.7	28.3
Thiamine + Nicotinic Acid	4.1	7.5	3.4	45.3
Riboflavin + Nicotinic Acid + Pantothenic Acid	3.7	6.3	2.6	41.2
Thiamine + Nicotinic Acid + Pantothenic Acid	3.7	6.7	3.0	44.7
Thiamine + Riboflavin + Nicotinic Acid + Pantothenic Acid	3.6	8.4	4.8	57.1

*Average of duplicate plots.

Whether the vitamins were applied singly to the plots or in various combinations, they always produced increases in yields. Riboflavin alone produced the smallest increase (23.2%) and when combined with nicotinic acid the increase over the previous period was 28.3.% Thiamine, pantothenic acid, and nicotinic acid produced increases of 39.0% or more than the previous yields of their respective plots. Combinations of twos and threes produced increases between 40.0% and 48.7% over the previous yields of these same plots respectively before treatments were started. When all four of the vitamins were combined the increase was 57.1%. The results of this experiment tend to show that increases in yields of mushrooms may be expected any time during the production period of the beds after applications of the B-vitamins have been started.

Since pyridoxine did not add much to the yields and biotin was difficult to secure, and both were expensive, they were omitted after the first trial.

ORGANIC MATERIALS

The organic materials, milorganite, BY-500, Kapost, and cyclone products were applied to duplicate plots at the rates listed in Table 10, and the results are presented in Table 10.

TABLE 10.—The effect on yields of mushrooms by adding certain organic materials to the compost

Treatment	Yield per square foot	Difference due to treatment	Percentage gain over check
	lb.	lb.	
Check	1 40		
Milorganite	2.10	.70	50
BY-500	2 00	.60	47
Kapost	1.93	.53	38
Cyclone product	1.92	.52	37

Milorganite and BY-500 produced the greatest increases in yield, which were approximately 50 percent greater than the average of the checks. Kapost and cyclone products produced similar quantities of mushrooms which were 38 and 37 percent respectively greater than the average of the checks. No other differences were observed.

THE EFFECT OF VARIOUS ORGANIC SUBSTANCES ON THE VITAMIN B-COMPLEX CONTENT OF MUSHROOMS[3]

This study includes the data from the assay of 250 samples of mushrooms. The first year's study was made with samples obtained from two commercial houses in the Cleveland area in which several vitamin carrying organic substances were used in the compost. The second year's study was made from samples grown in the Experiment Station's house in which various B-complex vitamins, singly or in combination, were distributed over the various beds. The first samples from the Station house were taken soon after the treatment (Table 11) and the second lot of samples (Table 12) were taken several weeks later in order to determine whether the beds were becoming exhausted or more built up in their vitamin content as evidenced by the vitamin content of the mushrooms.

All of the assays were made by standard micro-biological methods (18). The average results of the samples obtained from the commercial houses in the Cleveland area are shown in Table 11.

TABLE 11.—Vitamin content of mushrooms—Micrograms/gram, moisture free

Additions to Compost	Riboflavin	Nicotinic Acid	Pantothenic Acid	Thiamine
BY-500, average	24.1 ± 1.2	437	214	3.8
Milorganite, average	19.8 ± 0.9	428	205	4.0
Cyclone waste, average	18.3 ± 1.3	414	200	4.0 3.7 ± 0.1
Check plot—no treatment, average	18.5 ± 1.4	446	230	3.3

It is observed that there is a small difference between the values for riboflavin, nicotinic acid and pantothenic acid as influenced by the various additions to the compost. When the results are compared to the check plot (no treatment) only BY-500 which carries a high riboflavin content produced mushrooms of a higher riboflavin content. This difference appears to be significant. No other differences were noted.

EXPERIMENT STATION HOUSE

In the Station house the beds were sprinkled with solutions of the various vitamins and then harvested in a few days and dried and assayed. Not all plots were represented in this first collection. These data are

[3]Analyses by Dr. Charles H. Hunt, Animal Science Department, Ohio Agricultural Experiment Station.

shown in Table 12 and represent the individual beds replicated 4 to 9 times. When the results of the assay of the mushrooms from treated plots are compared with the check plot no consistency is shown which would indicate a possible later effect with additional vitamin treatment. It is observed that the highest (42.8 μg/gm) and lowest (29.2 μg/gm) riboflavin content were obtained from plots receiving riboflavin treatment in addition to nicotinic and pantothenic acids. The possibility of the inhibiting effect of nicotinic and pantothenic acid on the riboflavin content of the mushrooms is not ruled out. The thiamine, nicotinic acid and pantothenic acid varied considerably and when their probable errors are noted no significance can be attached to these values.

TABLE 12.—The effect of various B-vitamins on the vitamin B-complex content of mushrooms

Treatment—4 to 9 Replications	Riboflavin r/gr and Probable error	Thiamine r/gr	Nicotinic Acid r/gr	Pantothenic Acid r/gr
Check—no treatment	38.9 ± 6.6	8.1 ± 0.5	488 ± 25.8	212
Riboflavin	32.2 ± 3.8	7.6 ± 0.6	449	184
Thiamine	36.9 ± 1.9	6.7 ± 0.5	488	214
Thiamine + Riboflavin	42.8 ± 3.4	5.4 ± 0.5	499 ± 21.3	193
Thiamine + Riboflavin + Nicotinic Acid + Pantothenic Acid	31.6 ± 3.0	6.9 ± 0.4	496	215
Riboflavin + Pantothenic Acid + Nicotinic Acid	29.2 ± 0.6	6.4 ± 0.5	462 ± 27.0	177
Thiamine + Nicotinic Acid	34.3 ± 6.6	7.4 ± 0.6	466	156
Thiamine + Nicotinic Acid + Pantothenic Acid	38.6 ± 5.2	7.4 ± 0.4	493	187
Nicotinic Acid	37.5 ± 2.4	6.6 ± 0.7	486	205

After several weeks of vitamin treatment another collection of mushrooms was made. These data are presented in Table 13 and represent samples from 4 to 9 replications.

There again it is observed that the highest and the lowest (25.2 μg/gm) content were obtained from those plots which received riboflavin treatments. It is further observed that the mushrooms with the lowest riobflavin content were additionally treated with nicotinic acid and pantothenic acid. No such effect of nicotinic acid alone or with riboflavin alone were tested. These results are similar to those shown in Table 12.

TABLE 13.—The effect of various B-vitamin treatments on the vitamin B-complex content of mushrooms—Micrograms/gram. Moisture free

Plot	Riboflavin	Nicotinic Acid	Pantothenic Acid	Thiamine
Check	38.9	481	203	8.9
Thiamine	36.9	483	214	8.8
Riboflavin	32.2	449	184	7.9
Nicotinic Acid	37.5	479	194	7.6
Pantothenic Acid	35.0	474	173	7.7
Thiamine + Riboflavin	46.5	499	174	6.9
Riboflavin + Nicotinic Acid	37.9	477	181	7.3
Thiamine + Nicotinic Acid	34.3	467	156	7.5
Riboflavin + Nicotinic Acid + Pantothenic Acid	29.2	462	177	8.0
Thiamine + Nicotinic Acid + Pantothenic Acid	38.6	480	187	8.3
Thiamine + Nicotinic Acid + Pantothenic Acid + Riboflavin	25.2	473	205	8.6

Further calculations were made and the results show that the average riboflavin content of all samples at the beginning of treatment was 35.8 μg/gm. while later the content was 35.8 μg/gm. showing no change due to the prolonged use of the vitamin in mushroom culture. There is a possibility that the maturity of the samples studied may have had an influence on their vitamin content. In sampling this was not considered.

It is observed that the mushrooms grown in Experiment Station house have a higher vitamin content than those grown in the cooperative (Cleveland) houses. No explanation can be offered for these differences.

DISCUSSION

Throughout the work on the effect of adding solutions of pure B-complex vitamins to mushroom beds in production, it has been observed that yields have been increased. When picking was continued long enough (12 to 14 weeks) and the amounts of the vitamins large enough, yields have been greatly increased. In some commercial mushroom growing sections of the United States, the beds were kept in production for 12 to 14 weeks or more, without the use of vitamin supplements depending upon the ability of the growers to control temperature and humidity during the spring months. It was during such long periods as this that the added vitamin solutions did most good.

In other mushroom growing sections, it was common practice to discontinue picking after the yields began to decline following about 8 weeks of heavy picking. Then the growers removed the old compost and started again. Some investigators, Lambert (7), Reeve (9), and Stollar (13) followed this method in their studies of the effects of B-complex vitamins on the yield of mushrooms. They obtained increases in yields as a rule, but they found them not significant. It was the belief of the authors that 8 weeks of picking was not sufficient time for the vitamins to exert their full effects. Refer to the length of time stated above.

Several organic materials were also added to the compost at the time the beds were filled and run through the final heating period. These supplements were BY-500, BY-100, milorganite, cyclone products and Kapost. All produced satisfactory increases in yield except BY-100. After the first trial it was discontinued. The others were satisfactory to use, but when once mixed with the compost and the beds cased, another application could not be made, because the materials were dry and could not be incorporated in the compost without destroying the beds. BY-500 was the best of these supplements. It not only produced the largest yields, but also produced mushrooms with the highest riboflavin content of any of the organic supplements. (Note Tables *12* and *13*).

The chemical assay of the mushrooms shows, in general, that treating mushroom beds with B-complex vitamins in water solution did not increase the vitamin content of the mushrooms. The increase in riboflavin in the mushrooms from the BY-500 treatment was found to be significant. This seems to be due to the large amount of riboflavin in the product, and seems to reflect some ability of the mushroom to absorb it from the compost. (See Tables *12* and *13*).

Built-up poultry litter and corn steep liquor were not tried in this work, but they had been used commercially and reported by growers (Dean and Paddock) to be quite helpful for increasing yields of mushrooms when compared with similar beds, but without additions of these supplements.

PRACTICAL APPLICATIONS

The average commercial yields are about 1½ pounds of mushrooms per square foot. By using B-complex vitamins in water solutions as described, or supplements of organic materials high in B-complex vitamins in commercial mushroom houses yields could be increased by 30 to

50 percent. An increase of 30 percent would add considerable quantities of mushrooms without increasing bed areas. It is also economical to do because of low costs.

The amounts of the vitamins to apply per square foot of bed area are given in Table 14.

TABLE 14.—Amount of each B-complex vitamin to apply per square foot of bed area

Thiamine Hydrochloride	.5 mgm
Riboflavin	1.0 mgm
Nicotinic Acid	12.5 mgm
Pantothenic Acid	3.75 mgm

After the area of the bed has been calculated in square feet, the appropriate amounts are weighed out and mixed if they are to be applied as a mixture. Then they are dissolved in enough water to sprinkle them evenly over the bed.

Table 15 lists the costs of the vitamins per ounce and the area of bed to be covered by an ounce of the vitamins.

TABLE 15.—Cost of the four B-vitamins and areas one ounce will cover

B Vitamin	Cost* per Ounce	Area to Cover Square Foot
Thiamine Hydrochloride	$4.25	57,000
Riboflavin	2.85	28,500
Nicotinic Acid	.35	2,280
Calcium Pantothenate	2.85	7,600

*Research grade, October 1957. National Biochemicals Corporation, Cleveland, Ohio.

This research showed that more than one application of the vitamins should be made to get the greatest yields and also to have the picking season last from 12 to 14 weeks.

It is recommended that the first application be made before the bed is cased (Figs. 5 and 6) and then follow with applications every week to 10 days during the crop. Better results from the vitamins are usually had

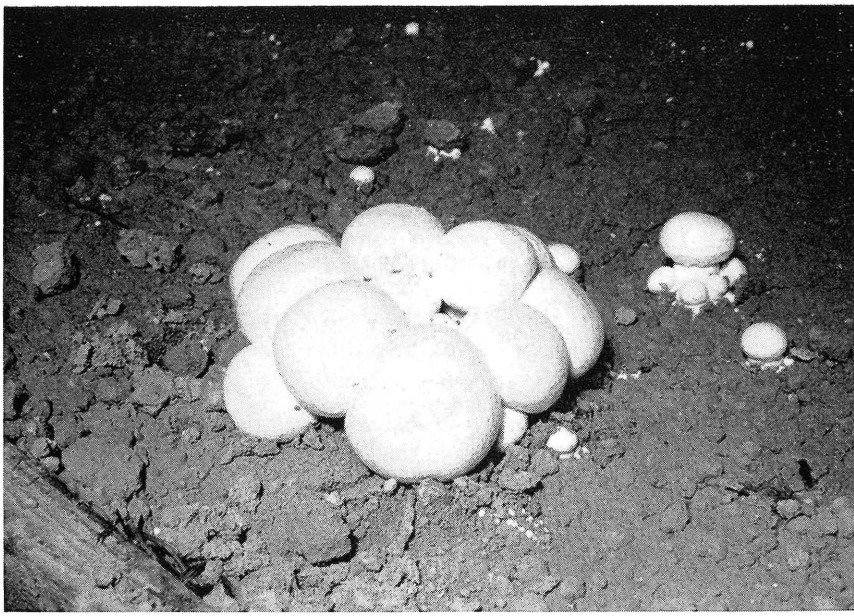

Fig. 5.—First flush of mushrooms appearing over the spot where a small portion of spawn was inserted in the compost and not treated with a vitamin. Compare with Figure 6.

with long cropping periods (12 to 14 weeks) than with short ones (7 to 8 weeks), because the compost seems to have enough of the vitamins to last during the first flushes. However, yields are larger when the vitamin treatments are started before casing than when delayed for several weeks after picking starts.

Other materials containing large amounts of the B-vitamins also increased mushroom yields in similar amounts. They were mixed with the compost as the beds were filled so they would go through the heating period. These are BY-500, milorganite, cyclone products and Kapost. BY-500** was used at the rate of 15 pounds per 100 square feet. Milorganite***, cyclone products*** and Kapost**** were used at the rate of 50 pounds per 100 square feet because their vitamin content was not so high. No doubt other materials could be used also, but so far they have not been tried experimentally.

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**BY–500, Commercial Solvents Corporation, Terre Haute, Indiana

***Milorganite and cyclone products, Milwaukee Sewerage Commission, Milwaukee, Wisconsin

****Kapost, Kalin Compost Company, Cleveland, Ohio

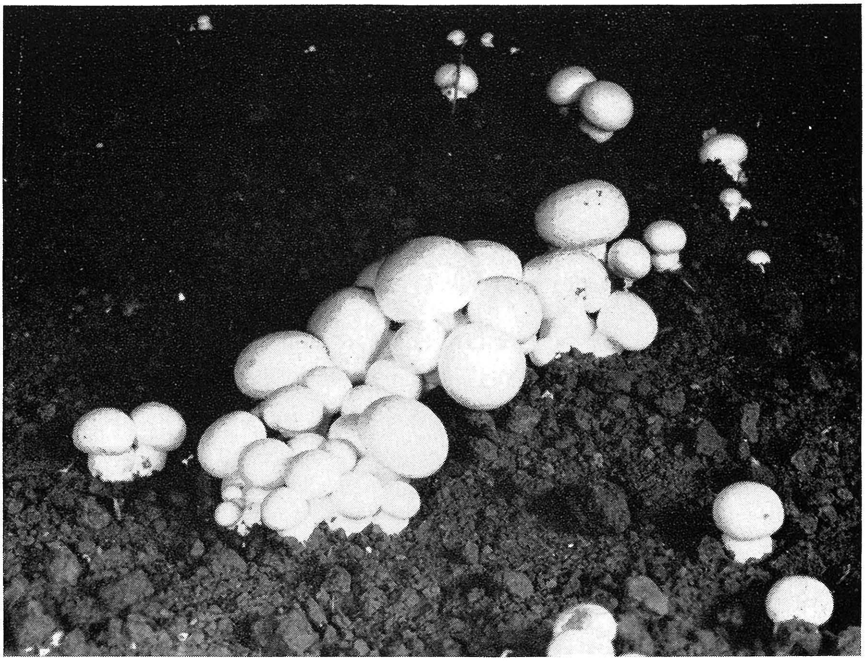

Fig. 6.—First flush of mushrooms appearing over the spot where a small portion of spawn was inserted in the compost and then followed by an application of thiamine in solution before the casing soil was put on the bed. Two weeks after casing the bed. (Compare with Fig. 5).

SUMMARY

1. After 8 to 10 weeks of heavy picking the yields of most commercial mushroom beds fall off rapidly.

2. High yields have not been maintained by supplementary nitrogen and mineral fertilizers.

3. Some increases in yields have been secured by more careful handling of the details of culture methods.

4. B-complex vitamins were tried to see if yields could be maintained at a higher level by their use. Those selected for trial were thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine and biotin.

5. All of these vitamins produced considerable increases in total yields.

6. Combinations of two, three, and more vitamins produced even greater increases except where pyridoxine and biotin were included. They did not increase yields.

7. Thiamine, riboflavin, nicotinic acid and pantothenic acid combined produced the largest increase in yield.

8. BY-500, BY-100, milorganite, cyclone products and Kapost were tried as supplementary organic materials having high B-complex vitamin contents. They all produced large increases in yield except BY-100. BY-500 was the most effective of this group of materials.

10. The B-vitamin content of mushrooms was not increased significantly by any of these supplements except where BY-500 was used alone. In this instance riboflavin was significantly increased.

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